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On the Reactions of Carbonyl Compounds with N-Salicylideneglycinato-aquo-copper (II) — Syntheses of β-Hydroxy α-Amino Acid from Glycine[1/]

by

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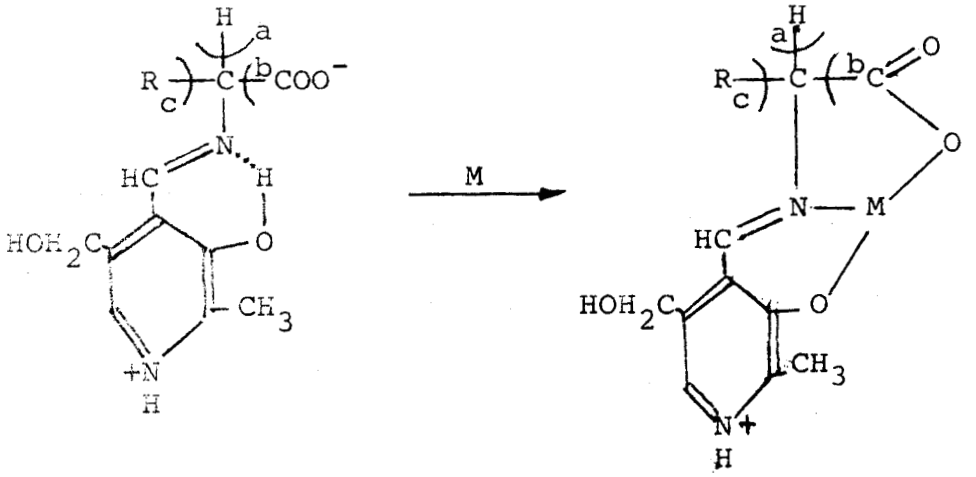
SUMMARY

Several forms of N-salicylideneglycinato-aquo-copper (II) were synthesized. These complexes were found to be the same in structure except for combining with water. Carbonyl compounds react rather easily with these complexes to form N-salicyliden β-hydroxy amino acid copper complex under conditions of aqueous solution, at room temperature (25° ± 1°) and at neutral to weakly alkaline pH. Threonine, β-phenylserine, β-hydroxyaspartic acid, and serine were synthesized. The yields dependent on reaction time and the ratios of threo and erythro isomers were studied. The reactions are similar to those non-enzymatically catalyzed by pyridoxal and the complexes could be regarded as the benzene analogues of pyridoxal-amino acid complexes.

It has been found that many naturally occurring enzymes contain pyridoxal-5-phosphate as a coenzyme[2]. Nonenzymatic reactions catalyzed by pyridoxal or pyridoxamine have been studied extensively by Metzler and Snell[3]. The chemistry of pyridoxal was reviewed by Snell[3], Westheimer[4], and Braunstein[5]. Aldimines (I) are formed from pyridoxal with amino acids, and their metal chelating compounds will be illustrated as (II) in Fig. 1[3c].



I II

Fig. 1

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[2] Some of the pyridoxal-containing enzymes are amino acid decarboxylase, transaminase, tryptophanase, kynureninase, tyrosinase, serine deaminase, cysteine desulfhydrase, and hydroxyamino acid aldolase.

[3] a) E. E. Snell, Special Lectures in Biochemistry, pp. 1-16, University College, London. H. K. Lewis, Distributors (1954-1955).
b) Vitamins and Hormones, 16, 78 (1960).
c) Chemical and Biological Aspects of Pyridoxal Catalysis, ed. E. E. Snell, P. M. Fasella, A. Braunstein, and A. Rossi Fanelli, p. 1. The Macmillan Company, New York, 1963.

[4] F. H. Westheimer, The Enzymes, ed. P. D. Boyer, H. Lardy, and K. Myrback, Vol. 1, p. 259, Academic Press, 1959.

[5] A. E. Braunstein, ibid., Vol 2, p. 113, Academic Press, 1960.

In both structures I and II, chemical bonds a, b, and c combined with the α-carbon atom of the amino acid could be weakened by the aldimine formation. In structure I, bond b might be the weakest and decarboxylation might take place. On the other hand, in structure II, the carboxyl group could be stabilized by the formation of a chelate, and bond c could be weakened. Metzler, Longenecker, Ikawa, and Snell[6,7] reported the reversible catalytic cleavage of β-hydroxy-α-amino acids using pyridoxal and metal ions by heating in aqueous solution. These nonenzymatic reactions catalyzed by pyridoxal seem to be similar in reaction mechanism to those catalyzed by enzymes which contain pyridoxal as a coenzyme. Ikawa and Snell[8] have studied the chemical properties of benzene analogs of pyridoxal with amino acids. They found 4-nitrosalicyaldehyde simulates pyridoxal in its reactions with several amino acids. 4-Nitrosalicylaldehyde catalyzes the dehydration of serine, the desulfhydration of cysteine, and the splitting of threonine to glycine as pyridoxal. In each of these reactions metal ions were found to be necessary. However, salicylaldehyde was found to be ineffective.

In this investigation, N-salicylideneglycinato-aquo-copper (II), which could be recognized as a benzene analog of pyridoxal-amino-metal complex (II), illustrated in Fig. 1 (R = H), was prepared. By the use of the N-salicylidenglycinato-copper (II)

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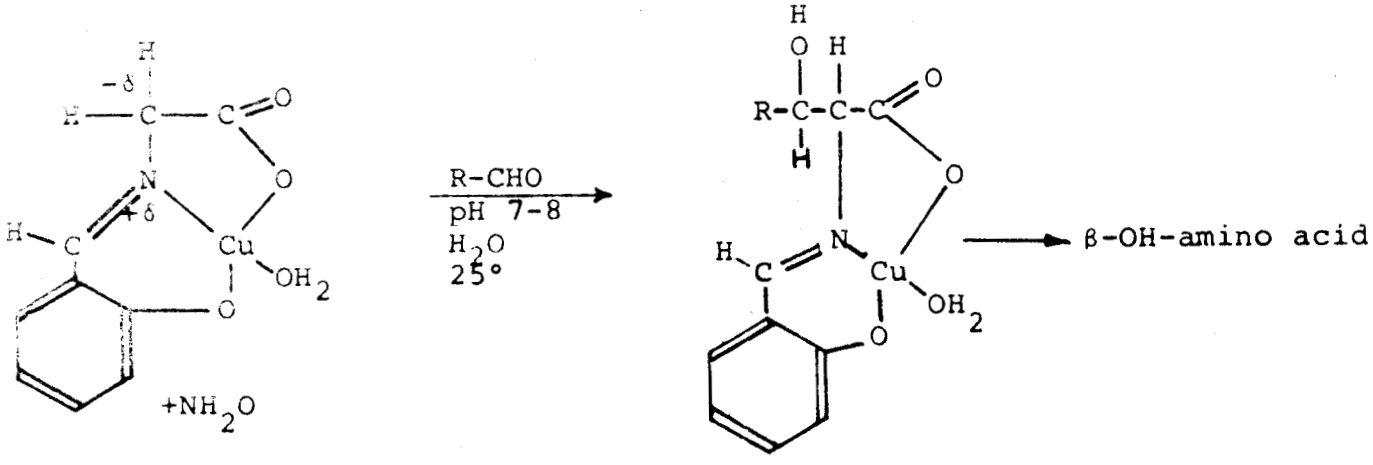
[6] D. E. Metzler, J. B. Longenecker, and E. E. Snell, J. Am. Chem. Soc., 75, 2786 (1953); ibid., 76, 639 (1954).

[7] D. E. Metzler, M. Ikawa, and E. E. Snell, ibid., 76, 648 (1954).

[8] M. Ikawa and E. E. Snell, ibid., 76, 653 (1954).

complex, the syntheses of various β-hydroxy α-amino acids have been studied.

The complex was first prepared in solution by Eichhorn and Marchand[9]. In their study, the coordination of copper (II) ion to the Schiff base formed from glycine and salicylaldehyde resulted in a stabilization of the Schiff base which would hydrolyze in the absence of a metal. Recently Nakahara[10] synthesized the complex in a crystalline state and he investigated the complex spectroscopically. Nakahara concluded that the complex is the copper (II) chelate of N-salicylideneglycine and the structure is the same as postulated by Eichhorn and Marchand[9] analytically and spectroscopically as illustrated below (Structure III, Fig. 2).



III IV

Fig. 2

[9] G. L. Eichhorn and N. D. Marchand, J. Am. Chem. Soc., 78, 2688 (1956).

[10] A. Nakahara, Bull. Chem. Soc. Japan, 32, 1195 (1959).

Nakahara prepared the complex in an acidic aqueous solution and obtained a yellow-green complex (crystal A-1). However, when we prepared the complex under neutral or weakly alkaline conditions (pH 7.5-8.0), different colored complexes were obtained. When the reaction mixture was at pH 8, dark blue crystals (crystal A-2) were obtained. These were recrystallized from water and ethanol and blue-green crystals were obtained (crystal A-3). Crystal A-3 was again recrystallized from the same solvent and dark green crystals were obtained (crystal A-4). Crystal A-4 seems to be a stable form in the recrystallization solvent system and the color, crystalline form, and infrared absorption spectra do not change by further recrystallization. According to the visible and long ultraviolet absorption spectra of these four complexes in sodium hydrogen carbonate solutions, they were the same and showed absorption maxima at 667 mμ and at 350 mμ. This suggests that the complexes have the same structure and are different in how they combine with water in quality or in its amount. Quantitative analyses of glycine in these complexes were carried out by the use of the automatic amino acid analyzer after decomposition of complexes by hydrochloric acid. These results indicate the possible molecular formulas of complexes A-1, A-2, A-3, and A-4 which are shown in Table I.

TABLE I

Possible Molecular Formula of

N-Salicylideneglycinato-aquo-Cu (II)

		Mol. weight, found	Possible mol. formula	Calcd. mol. weight
A-1	yellow-green	282.7	$C_9H_7NO_3Cu.2\ H_2O$	276.7
A-2	dark blue	409.4	$C_9H_7NO_3Cu.8\ H_2O$	402.9
A-3	blue-green	350.7	$C_9H_7NO_3Cu.5\ H_2O$	348.8
A-4	dark green	276.7	$C_9H_7NO_3Cu.2\ H_2O$	276.7

The reactions of the complexes with carbonyl compounds were carried out under conditions similar to physiological conditions: aqueous solution at pH 7.0-8.0 and at room temperature (25° ± 1°) (Fig. 2). Acetaldehyde and the complex resulted in a mixture of N-salicyliden Cu (II) complex of threonine and of glycine. Observed results of the threonine syntheses are summarized in Table II. The results show that complex A-4 is more reactive than A-1 and the yield of (threonine and allothreonine) reached almost 90% based on the starting complex, A-4. The ratios of threonine and allothreonine were measured colorimetrically by a Beckmann-Spinco Analytrol after paper chromatography[11]. The ratios of threonine and allothreonine in the products were in a relatively narrow range in all reactions, threonine 31-34%, allothreonine 66-69%. From this reaction mixture, allothreonine was isolated (see Experimental section).

[11] T. L. Hardy and P. O. Holland, Chem. & Ind., 1954, 517.

TABLE II

Formation of Threonine

Reaction time, hr	A-1			A-4		
	Thr,%[a]	Gly,%[a]	(Yield) Thr,%[b]	Thr,%[a]	Gly,%[a]	(Yield) Thr,%[b]
24	60.7	39.3	(56.3)	95.6	4.4	(84.3)
48	62.2	37.8	(58.5)	97.7	2.3	(87.5)
72	63.5	36.5	(55.8)	95.5	4.5	(89.2)
96	63.9	36.1	(52.8)	97.7	2.3	(79.1)
120	65.2	34.8	(51.3)	97.2	2.8	(72.8)

[a] Amino acid compositions of the reaction mixture are shown in molar percent.

[b] Calculated yields are based on the starting complexes A-1 and A-4.

In a similar way, phenylserine formation from complex A-1 and A-4 with benzaldehyde was studied under similar conditions. Summarized results are listed in Table III.

TABLE III

Formation of β-Phenylserine

Reaction time, hr	A-1			A-4		
	Phe-ser,% [a]	Gly,% [a]	(Yield) Phe-ser,% [b]	Phe-ser,% [a]	Gly,% [a]	(Yield) Phe-ser,% [b]
24	42.7	57.3	(38.9)	54.2	45.8	(42.5)
48	45.2	54.8	(42.5)	59.2	40.8	(45.1)

[a] Amino acid compositions of the reaction products are expressed in molar percent.

[b] Yields are calculated based on the starting complexes A-1 and A-4.

The ratios of threo- and erythro-phenylserine of the product were measured colorimetrically after paper chromatographic separation[12]. A larger amount of threo-phenylserine was synthesized in the reactions: A-1, threo 77.4-82.2%, erythro 22.6-17.8%; A-4, threo 79.2-84.1%, erythro 20.8-15.9%. From the reaction mixture, pure threo-phenylserine was isolated (see Experimental section).

β-Hydroxyaspartic acid was prepared from the complexes and glyoxylic acid. In this reaction, however, refluxing conditions were employed. Products were analyzed to determine the yields and amino acid compositions of the products by an automatic amino acid analyzer. Table IV shows the summarized results of β-hydroxyaspartic acid formation.

Synthesis of serine from the complexes was studied. The yield of serine, however, was found to be very low under the reaction conditions (pH 7-8, at room temperature in aqueous solution). Summarized results are shown in Table V.

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[12] K. N. F. Shaw and S. W. Fox, J. Am. Chem. Soc., 75, 3421 (1953).

TABLE IV

Formation of β-Hydroxyaspartic Acid

Reaction (min. of refluxing)	(Yield) β-OH-Asp, % [a]	Amino acid composition of the reaction product [b]		
		Threo-β-OH-Asp	Erythro-β-OH-Asp %	Gly %
10	49.4	43.0	24.5	32.5
10 (without $NaHCO_3$)	50.6	42.9	23.3	33.8
15	50.9	42.9	23.5	33.6
60	28.4	29.3	13.2	57.5

[a] Calculated yields are based on the starting complex A-4.

[b] Amino acid compositions of the reaction mixtures are expressed in molar percent.

TABLE V

Formation of Serine

Reaction time (hr)	Ser, % [a]	Gly, % [a]	(Yield) Ser, % [b]
24	4.6	95.4	(2.6)
120	4.7	95.3	(1.1)

[a] Amino acid compositions of the reaction products are expressed in molar percent.

[b] Calculated yields are based on the starting complex A-4.

EXPERIMENTAL[13]

N-Salicylideneglycinato-copper (II) — N-Salicylideneglycinato-copper (II) complex (A-1) was prepared by the same method described by Nakahara, and a yellow-green complex was obtained.

Other preparations of the complex are as follows: glycine, 7.50 g (0.10 mole), and sodium hydroxide, 8.0 g (0.20 mole), were dissolved in 30 ml of water and the temperature brought to 40°. To this solution, 12.0 ml (0.1 mole) of salicylaldehyde in 10 ml of ethanol was added. Then a hot solution of 19.9 g (0.1 mole) cupric acetate monohydrate in 80 ml of water was added to the reaction mixture. After 10 minutes of agitation, a mixture of ethanol (120 ml) and ether (120 ml) was added to complete the precipitation. After one hr of standing at room temperature, the precipitated crystals were filtered. Yield, 29.0 g (71.9%). The crystals were dark blue (crystal A-2). These crystals (A-2) were recrystallized from water and ethanol (1:1) and blue-green crystals were obtained (crystal A-3), yield 21.1 g (60.3%). Crystals (A-3) were recrystallized again from water and ethanol (1:2) and dark green crystals were obtained (crystal A-4), yield 13.4 g (48.3%). Elemental analysis of A-4 is as follows:

Anal. Calcd. for $C_9H_{11}NO_5Cu.(2\ H_2O)$: C, 39.06; H, 4.01; N, 5.06. Found: C, 39.18; H, 3.90; N, 5.07.

The glycine content of these complexes, A-1, A-2, A-3, and A-4 was measured. A known amount of these complexes was decomposed

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[13] Elemental analyses were done by Micro-Tech Laboratories, Skokie, Illinois. Amino acid analyses were carried out by Phoenix K-5000 automatic amino acid analyzer.

with 1 N hydrochloric acid and the solutions were applied to the Phoenix K-5000 automatic amino acid analyzer to determine glycine content.

Visible and long ultraviolet absorption spectra of A-1, A-2, A-3, and A-4 were measured. Each of the complexes (5.0 mg) was dissolved in 10 ml of water containing 84 mg of sodium hydrogen carbonate. Absorption maxima: 667 mμ and 350 mμ.

Threonine and allothreonine — Complexes (A-1) and (A-4), 64 mg each, 250 μmole, were suspended in 4 ml of water and 84 mg of sodium hydrogen carbonate and 0.4 ml of acetaldehyde were each added to the suspensions. The mixtures were shaken for 24, 48, 72, 96, and 120 hr at room temperature (25°). Then 1 ml of 6 N hydrochloric acid was added to each of the samples to decompose the copper complex, and the resulting amino acids were analyzed by the amino acid analyzer. Results are summarized in Table II.

Threonine and allothreonine were resolved by paper chromatography[11,12]. After staining with ninhydrin, the color density was measured by a Beckman-Spinco Analytrol intensitometer. R_f values of amino acids are as follows: threonine, 0.54; allothreonine, 0.41; glycine, 0.15.

Isolation of allothreonine — Complex A-4 (2.76 g, 0.01 mole) was suspended in 120 ml of water and 3.3 g of sodium hydrogen carbonate and 16 ml of acetaldehyde were added. This mixture was shaken for 24 hr at room temperature. After reaction, the excess acetaldehyde was removed in vacuo. This reaction mixture was

acidified with 6 N hydrochloric acid. Then the liberated salicylaldehyde was extracted with ether. The aqueous solution was concentrated to dryness in vacuo. The residue was extracted with absolute ethanol. The resulting sodium chloride was removed by filtration. Ethanol was removed under reduced pressure. The residue was dissolved in 5 ml of water, then applied to a column of Dowex 50 x 2 (H-form, 100-200 mesh, 2 x 20 cm) and washed with water to neutrality, then eluted with 1 N ammonia. The effluent was evaporated in vacuo and treated with active carbon. This solution was again concentrated and 95% ethanol was added. After being kept in a refrigerator overnight, the crystals were filtered. The crystals, 450 mg (38%), were recrystallized from water and ethanol three times. Yield, 340 mg (29%). mp 239° dec. By paper chromatography, the crystals were identified as allothreonine containing trace amounts of glycine. Comparison of the infrared spectrum of the allothreonine agreed with authentic allothreonine in all absorption maxima.

Anal. Calcd. for $C_4H_9NO_3$: C, 40.33; H, 7.62; N, 11.76. Found: C, 40.31; H, 7.36; N, 11.90.

threo- and erythro-Phenylserine — The complexes (A-1) and (A-4), (69 mg each, 250 mole), were suspended in a mixture of 3 ml water and 3 ml 95% ethanol. To these were added 84 mg of sodium bicarbonate and 0.75 ml of benzaldehyde. These mixtures were shaken for 24 hr and 48 hr at room temperature. 6 N Hydrochloric acid, 1 ml, was added to this reaction mixture to decompose the copper complex. The reaction mixture was then analyzed by the amino acid analyzer.

threo- and erythro-Phenylserine were resolved by paper chromatography[12]. The color density was measured after staining with ninhydrin by a Beckman-Spinco Analytrol intensitometer. R_f Values of amino acids are as follows: threo-phenylserine, 0.42; erythro-phenylserine, 0.27; glycine, 0.12. Results are summarized in Table III.

Isolation of threo- and erythro-phenylserine — Complex A-4 (2.76 g, 0.01 mole) was suspended in a mixture of 60 ml of water and 60 ml of 95% ethanol. To this was added 3.3 g of sodium hydrogen carbonate and 29 ml of benzaldehyde. The mixture was shaken for 24 hr at room temperature. The reaction mixture was acidified with 6 N hydrochloric acid. The salicylaldehyde and excess benzaldehyde were extracted with ether. The aqueous solution was evaporated to dryness in vacuo. The residue was extracted with absolute ethanol and insoluble sodium chloride was removed by filtration. The ethanolic solution was evaporated to dryness. The residue was dissolved in 5 ml of water and this was applied to a column of Dowex 50 x 2 (H-form, 100-200 mesh, 2 x 20 cm). The column was washed with water until the effluent water became neutral. Then the amino acid was eluted with 1 N ammonia. Fractions containing the amino acid were combined and concentrated to about 5 ml, and were kept in a refrigerator overnight. Precipitated crystals were filtered and were recrystallized from water and 95% ethanol twice. Yield, 480 mg (24%). mp 195° dec.

Anal. Calcd. for $C_9H_{13}NO_4$: C, 54.26; H, 6.58; N, 7.03. Found: C, 54.52; H, 6.54; N, 7.33.

These crystals were identified as pure threo-phenylserine by paper chromatography[12] and by the IR spectrum.

The mother liquor, from which the threo-phenylserine was already removed, was evaporated to dryness in vacuo. This was dissolved in 1.4 ml of hot water and 2 ml of hot dioxane was added. Crystallization began after a few minutes. The suspension was kept in a refrigerator overnight and the crystals were collected by filtration. The crystals were washed with a mixture of water and dioxane (1:1). These crystals, 230 mg (10%), were recrystallized from water and dioxane three times. Yield, 170 mg (7.6%). mp 192° dec. These crystals were found to be erythro-phenylserine dioxane adduct and did not contain threo-phenylserine. However, they contained a small amount (3%) of glycine. Comparison of the spectrum of these crystals with the authentic dioxane adduct of erythro-phenylserine showed no difference.

threo- and erythro-β-Hydroxyaspartic acid — Complex A-4 (2.76 g, 100 μmole), sodium glyoxylate (9.6 mg, 100 μmole), and sodium hydrogen carbonate (42 mg) were dissolved in 10 ml of water and refluxed for 10, 15, and 60 min. These solutions were analyzed after decomposing the copper complex by hydrochloric acid by the amino acid analyzer. Effluent volume of amino acids are as follows: threo-isomer, 52.5 ml; erythro-isomer, 63.0 ml; glycine, 116.7 ml.

Serine — Complex A-4 (69 mg, 250 μmole) was suspended in 4 ml of water, then 84 mg of sodium hydrogen carbonate and 2 ml of 35% formaldehyde solution were added. These mixtures were shaken for

24 hr and 120 hr at room temperature. The reaction products were analyzed by the amino acid analyzer. Results are shown in Table V.

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